

WHAT IS CLAIMED IS:

1. A method for introducing a double-stranded target DNA into a vector comprising culturing a bacterial cell that expresses a functional recombinase, said bacterial cell
5 containing (a) the target DNA comprising a first double-stranded terminus and a second double-stranded terminus, and (b) a vector DNA comprising, in the following order along the vector DNA strand: (i) a first double-stranded homology arm (ii) an origin of replication; and (iii) a second double-stranded homology arm, such that the sequence of a
10 vector DNA strand of the first homology arm is homologous to the sequence of a target DNA strand of the first terminus, and the sequence of a vector DNA strand of the second homology arm is homologous to the sequence of the target DNA strand of the second terminus, such that the target DNA is inserted into the vector DNA between the homology arms.
- 15 2. A method for making a recombinant DNA molecule comprising:
 - a) introducing a double-stranded vector into a cell, said cell containing a double-stranded target DNA and expressing a bacterial recombinase, said vector comprising an origin of replication and two homology arms, in the
20 following order from 5' to 3' along a vector DNA strand: a first homology arm, one strand of the origin of replication, and a second homology arm; , said target DNA comprising a target DNA sequence and two termini, in the following order, from 3' to 5' along a target DNA strand: a first terminus, the target DNA sequence, and a second terminus,
25 such that the sequence of the first homology arm on said vector DNA strand is homologous to the sequence of the first terminus on said target DNA strand, and the sequence of the second homology arm on said vector DNA strand is homologous to the sequence of the second terminus on said target DNA strand; and
 - b) subjecting the cell to conditions that allow intracellular homologous
30 recombination to occur.

3. A method for making a recombinant DNA molecule comprising:
- a) introducing a double-stranded vector and first and second double-stranded oligonucleotides into a cell, said cell containing a double-stranded target DNA and expressing a bacterial recombinase,
- 5 said vector comprising an origin of replication and two double-stranded homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication, and a second homology arm;
- 10 said target DNA comprising a target DNA sequence and two double-stranded termini, in the following order, from 3' to 5' along a target DNA strand: a first terminus, a target DNA sequence, and a second terminus;
- 15 said first oligonucleotide comprising a first oligonucleotide DNA strand comprising, in the following order, from 3' to 5': a first nucleotide sequence and a second nucleotide sequence, said first nucleotide sequence being homologous to the nucleotide sequence of the first homology arm on said vector DNA strand, and said second nucleotide sequence being homologous to the nucleotide sequence of the first terminus on said target DNA strand;
- 20 said second oligonucleotide comprising a second oligonucleotide strand comprising, in the following order, from 3' to 5', a third nucleotide sequence and a fourth nucleotide sequence, said third nucleotide sequence being homologous to the nucleotide sequence of the second homology arm on said vector DNA strand and said fourth nucleotide sequence being homologous to the nucleotide sequence of the second terminus on said target DNA strand;
- 25 and
- b) subjecting the cell to conditions that allow intracellular homologous recombination to occur.
4. A method for making a recombinant DNA molecule comprising:
- a) introducing a double-stranded target DNA molecule into a cell, said cell
- 30 containing a vector and expressing a bacterial recombinase,

said target DNA comprising a target DNA sequence and two double-stranded termini, in the following order, from 3' to 5' along a target DNA strand: a first terminus, a target DNA sequence, and a second terminus;
said vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication and a second homology arm;
such that the sequence of the first homology arm on said vector DNA strand is homologous to the sequence of the first terminus on said target DNA strand, and the sequence of the second homology arm on said vector DNA strand is homologous to the sequence of the second terminus on said target DNA strand; and
b) subjecting the cell to conditions that allow intracellular homologous recombination to occur.

5. A method for making a recombinant DNA molecule comprising:

a) introducing a double-stranded target DNA molecule and a first and second double-stranded oligonucleotide into a cell, said cell containing a vector and expressing a bacterial recombinase, said target DNA comprising a target DNA sequence and two termini, in the following order, from 3' to 5' along a target DNA strand: a first terminus, a target DNA sequence, and a second terminus;
said first oligonucleotide comprising a first oligonucleotide DNA strand comprising, in the following order, from 3' to 5': a first nucleotide sequence and a second nucleotide sequence, said first nucleotide sequence being homologous to the nucleotide sequence of the first homology arm on said vector DNA strand, and said second nucleotide sequence being homologous to the nucleotide sequence of the first terminus on said target DNA strand;
said second oligonucleotide comprising a second oligonucleotide strand comprising, in the following order, from 3' to 5', a third nucleotide sequence and a fourth nucleotide sequence, said third nucleotide sequence being

homologous to the nucleotide sequence of the second homology arm on said vector DNA strand and said fourth nucleotide sequence being homologous to the nucleotide sequence of the second terminus on said target DNA strand; and

- 5 said vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication and a second homology arm; and
- b) subjecting the cell to conditions that allow intracellular homologous recombination to occur.

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6. A method for making a recombinant DNA molecule comprising:

- a) introducing a double-stranded vector and a double-stranded target DNA into a cell expressing a bacterial recombinase,
- 15 said vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication and a second homology arm,
- 20 said target DNA comprising a target DNA sequence and two termini, in the following order, from 3' to 5' along a target DNA strand: a first terminus, a target DNA sequence; and a second terminus;
- 25 such that the nucleotide sequence of the first homology arm on said vector DNA strand is homologous to the nucleotide sequence of the first terminus on said target DNA strand, and the nucleotide sequence of the second homology arm on said vector DNA strand is homologous to the sequence of the second terminus on said target DNA strand; and
- b) subjecting the cell to conditions that allow intracellular homologous recombination to occur.

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7. A method for making a recombinant DNA molecule comprising:
- a) introducing a double-stranded vector, a double-stranded target DNA molecule, and a first and second double-stranded oligonucleotide into a cell expressing a bacterial recombinase,
- 5 said vector comprising an origin of replication and two double-stranded homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication and a second homology arm;
- 10 said target DNA comprising target DNA sequence and two double-stranded termini, in the following order, from 3' to 5' along a target DNA strand: a first terminus, a target DNA sequence, and a second terminus;
- 15 said first oligonucleotide comprising a first oligonucleotide DNA strand comprising, in the following order, from 3' to 5': a first nucleotide sequence and a second nucleotide sequence, said first nucleotide sequence being homologous to the nucleotide sequence of the first homology arm on said vector DNA strand, and said second nucleotide sequence being homologous to the sequence of the first terminus on said target DNA strand;
- 20 said second oligonucleotide comprising a second oligonucleotide strand comprising, in the following order, from 3' to 5', a third nucleotide sequence and a fourth nucleotide sequence, said third nucleotide sequence being homologous to the nucleotide sequence of the second homology arm on said vector DNA strand and said fourth nucleotide sequence being homologous to the nucleotide sequence of the second terminus on said target DNA strand;
- 25 and
- b) subjecting the cell to conditions that allow intracellular homologous recombination to occur.
8. The method of Claim 6 wherein the host cell further contains a nucleotide sequence encoding a site-specific recombinase operatively linked to a promoter, and the vector further
- 30 comprises a first and second recognition site for the site-specific recombinase, a first

recognition site located outside the first and second homology arms, and a second site-specific recombinase recognition site located inside the first and second homology arms; and during or after step b), inducing expression of the site-specific recombinase.

5 9. The method of Claim 7 wherein the host cell further contains a nucleotide sequence encoding a site-specific recombinase operatively linked to a promoter, and the vector further comprises a first and second recognition site for the site-specific recombinase, a first recognition site located outside the first and second homology arms, and a second site-specific recombinase recognition site located inside the first and second homology arms;
10 and during or after step b), inducing expression of the site-specific recombinase.

10. The method of Claim 6 wherein the host cell further contains a nucleotide sequence encoding a site-specific endonuclease operatively linked to a promoter, and the vector further comprises a recognition site for the site-specific endonuclease located inside the
15 first and second homology arms; and during or after step b), inducing expression of the site-specific endonuclease.

11. The method of Claim 7 wherein the host cell further contains a nucleotide sequence encoding a site-specific endonuclease operatively linked to a promoter, and the vector
20 further comprises a recognition site for the site-specific endonuclease located inside the first and second homology arms; and during or after step b), inducing expression of the site-specific endonuclease.

12. The method of any one of Claims 2-11 wherein the vector further comprises a
25 selectable marker located outside the first and second homology arms, such that the vector comprises, in either of the following two orders from 5' to 3' along a vector DNA strand: i) the first homology arm, the selectable marker, the origin of replication and the second homology arm, or ii) the first homology arm, the origin of replication, the selectable marker, and the second homology arm.

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13. The method of Claim 12 wherein the selectable marker confers antibiotic resistance to the cell containing the vector.
14. The method of any one of Claims 2-11 wherein the bacterial recombinase is RecE/T or Red α / β recombinase or both RecE/T and Red α / β recombinases.
15. The method of any one of Claims 2-11 wherein the cell is a bacterial cell.
16. The method of any one of Claims 2-11 wherein the cell is an *E. coli* cell.
17. The method of any one of Claims 2-11 wherein the cell is a eukaryotic cell that recombinantly expresses RecE/T and/or Red α / β recombinase.
18. The method of any one of Claims 2-11 which further comprises isolating a recombinant DNA molecule that comprises the target DNA inserted into the vector.
19. A double-stranded DNA vector useful for directed cloning or subcloning of a target DNA molecule of interest, said vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication and a second homology arm; such that the nucleotide sequence of the first homology arm on a first vector DNA strand is homologous to the sequence of the first terminus on a first target DNA strand, and the nucleotide sequence of the second homology arm on the first vector DNA strand is homologous to the nucleotide sequence of the second terminus on the first target DNA strand.
20. The vector of Claim 19 wherein the origin of replication is a bacterial origin of replication.
21. The vector of Claim 19 wherein the origin of replication functions in *E. coli*.

22. The vector of Claim 19 wherein the origin of replication functions in a mammalian cell.

23. A cell comprising a double-stranded DNA vector useful for directed cloning or
5 subcloning of a target DNA molecule of interest, said vector comprising an origin of
replication and two homology arms, in the following order from 5' to 3' along a vector DNA
strand: a first homology arm, the origin of replication and a second homology arm; such
that the nucleotide sequence of the first homology arm on a first vector DNA strand is
homologous to the sequence of the first terminus on a first target DNA strand, and the
10 nucleotide sequence of the second homology arm on the first vector DNA strand is
homologous to the nucleotide sequence of the second terminus on the first target DNA
strand.

24. The cell of Claim 23 which is a bacterial cell.

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25. A kit useful for directed cloning or subcloning of a target DNA molecule comprising
in one or more containers:

- 20 a) a double-stranded DNA vector useful for directed cloning or subcloning of a
target DNA molecule of interest, said vector comprising an origin of
replication and two homology arms, in the following order from 5' to 3'
along a vector DNA strand: a first homology arm, the origin of replication
and a second homology arm; such that the nucleotide sequence of the first
homology arm on a first vector DNA strand is homologous to the sequence
of the first terminus on a first target DNA strand, and the nucleotide
25 sequence of the second homology arm on the first vector DNA strand is
homologous to the nucleotide sequence of the second terminus on the first
target DNA strand; and
- b) a cell containing a bacterial recombinase.

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26. The kit of Claim 25 wherein the homology arms have sequence homology to a BAC, PAC, lambda, plasmid or YAC based cloning vector.
27. The kit of Claim 25 wherein the first and second double-stranded oligonucleotide
5 have nucleotide sequence homology to a BAC, PAC, lambda, plasmid or YAC based cloning vector.
28. A kit useful for directed cloning or subcloning of a target DNA molecule comprising in one or more containers:
- 10 a) A double-stranded DNA vector useful for directed cloning and subcloning of a target DNA molecule of interest, said vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication and a second homology arm; and
- 15 b) a first double-stranded oligonucleotide comprising a first oligonucleotide DNA strand comprising, in the following order, from 3' to 5': a first sequence and a second sequence, said first nucleotide sequence being homologous to the nucleotide sequence of the first homology arm on said vector DNA strand, and said second nucleotide sequence being homologous
20 to the nucleotide sequence of a first terminus on a target DNA strand;
- c) a second double-stranded oligonucleotide comprising a second oligonucleotide strand comprising, in the following order, from 3' to 5': a third nucleotide sequence and a fourth nucleotide sequence, said third
25 nucleotide sequence being homologous to the nucleotide sequence of the second homology arm on said vector DNA strand and said fourth nucleotide sequence being homologous to the nucleotide sequence of a second terminus on said target DNA strand; and
- d) a cell containing a bacterial recombinase.
- 30 29. The kit of Claim 25 or 28 wherein the cell is an *E. coli* cell.

30. The kit of Claim 25 or 28 wherein the cell is a frozen cell competent for uptake of DNA.

31. A kit useful for directed cloning or subcloning of a target DNA molecule comprising
5 in one or more containers:

- a) a double-stranded DNA vector useful for directed cloning and subcloning of a target DNA molecule of interest, said vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication
10 and a second homology arm;
- b) a first double-stranded oligonucleotide comprising a first oligonucleotide DNA strand comprising, in the following order, from 3' to 5': a first nucleotide sequence and a second nucleotide sequence, said first nucleotide sequence being homologous to the nucleotide sequence of the first homology
15 arm on said vector DNA strand, and said second nucleotide sequence being homologous to the nucleotide sequence of a first terminus on a target DNA strand; and
- c) a second double-stranded oligonucleotide comprising a second oligonucleotide strand comprising, in the following order, from 3' to 5': a
20 third nucleotide sequence and a fourth nucleotide sequence, said third nucleotide sequence being homologous to the nucleotide sequence of the second homology arm on said vector DNA strand and said fourth sequence being homologous to the nucleotide sequence of a second terminus on said target DNA strand.

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32. The kit of Claim 25, 28, or 31 wherein the DNA vector is purified.

33. The kit of Claim 28 or 31 wherein the DNA vector, the first double-stranded oligonucleotide, and the second double-stranded oligonucleotide are purified.

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34. The kit of Claims 25, 28, or 31 wherein the target DNA molecule comprises bacterial, viral, parasite, or protozoan DNA.
35. The kit of Claims 25, 28, or 31 wherein the target DNA molecule comprises a
5 genetic mutation or polymorphism known or suspected to be associated with a disorder or disease.
36. The kit of any one of Claims 25-29 wherein the bacterial recombinase is RecE/T or Red α / β recombinase or both RecE/T and Red α / β recombinases.
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37. The method of any one of Claims 2-11 wherein the target DNA is known or suspected to be associated with a disorder or disease when genetically mutated.
38. The method of any one of Claims 2-11 wherein the target DNA is a bacterial, viral,
15 parasite, or protozoan DNA.
39. The method of Claims 2, 4, 6, 8, or 10 which further comprises detecting a recombinant DNA molecule that comprises the target DNA inserted into the vector.
- 20 40. The method of Claims 3, 5, 7, 9, or 11 which further comprises detecting a recombinant DNA molecule that comprises the target DNA inserted into the vector.
41. A method of detecting the presence of an infectious agent comprising carrying out the method of Claim 39, wherein the target DNA is derived from a patient suspected of
25 having the infectious disease, and the sequences of the first and second homology arms are homologous to the sequences present in DNA of the infectious agent.
42. A method of detecting the presence of an infectious agent comprising carrying out the method of Claim 40, wherein the target DNA is derived from a patient suspected of
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having the infectious disease, and said second and fourth nucleotide sequences are homologous to sequences present in DNA of the infectious agent.

43. The method of Claim 41 or 42 wherein the infectious agent is a virus, bacteria,
5 protozoa, fungus, or parasite.

44. A method of detecting the presence of a genetic condition, disease, disorder, or polymorphic trait comprising carrying out the method of Claim 39, wherein the target DNA is derived from a patient suspected of having a genetic condition, disease, disorder, or
10 polymorphic trait, and the sequence of the first homology arm is homologous to the sequence upstream from a site known or suspected to be associated with the genetic condition, disease, disorder, or polymorphic trait, and the sequence of the second homology arm is homologous to the sequence downstream from a site known or suspected to be associated with the genetic condition, disease, disorder, or polymorphic trait.

15 45. A method of detecting the presence of a genetic condition, genetic disease, genetic disorder, or polymorphic trait comprising carrying out the method of Claim 40, wherein the target DNA is derived from a patient suspected of having the genetic condition, genetic disease, genetic disorder, or polymorphic trait, and the sequence of the first double-stranded
20 oligonucleotide is homologous to the sequence upstream from a site known or suspected to be associated with the genetic condition, genetic disease, genetic disorder, or polymorphic trait, and the sequence of the second double-stranded oligonucleotide is homologous to the sequence downstream from a site known or suspected to be associated with the genetic condition, genetic disease, genetic disorder, or polymorphic trait.

25 46. The method of Claims 44 or 45 wherein the genetic condition, genetic disease, genetic disorder, or polymorphic trait is or predisposes the patient to cancer, asthma, arthritis, drug resistance, drug toxicity, or a neural, neuropsychiatric, metabolic, muscular, cardiovascular, or skin condition, disease or disorder.

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47. The vector of Claim 19 wherein the vector further comprises a selectable marker located outside the first and second homology arms, such that the vector comprises, in either of the following two orders from 5' to 3' along a vector DNA strand: i) the first homology arm, the selectable marker, the origin of replication and the second homology arm, or ii) the
5 first homology arm, the origin of replication, the selectable marker, and the second homology arm.

48. The vector of Claim 47, wherein the sequence of the vector does not contain one or more direct repeats of a sequence of at least five or more nucleotide base pairs either 5' or 3'
10 to both the origin of replication and the selectable marker.

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